

What is claimed is:

- 1 A device comprising a high density array of molecules capable of interrogation and immobilised on a solid planar surface, wherein the array allows the molecules to be individually resolved by optical microscopy, and wherein each molecule is immobilised by covalent bonding to the surface, other than at that part of each molecule that can be interrogated.
- 2 A device according to claim 1, wherein the covalent bonding to the surface is without an intermediate microsphere.
- 3 A device according to claim 1, wherein fewer than 50% of the arrayed molecules are the same.
- 4 A device according to claim 1, wherein adjacent molecules of the array are separated by a distance of at least 10nm.
- 5 A device according to claim 4, wherein the molecules are separated by a distance of at least 100nm.
- 6 A device according to claim 4, wherein the molecules are separated by a distance of at least 250nm.
- 7 A device according to claim 1, having a density of from 10^6 to 10^9 molecules per cm^2 .
- 8 A device according to claim 7, wherein the density is from 10^7 to 10^8 molecules per cm^2 .
- 9 A device according to claim 1, wherein the molecules are polynucleotides immobilised to the solid support via the 5' terminus, the 3' terminus or via an internal nucleotide.
- 10 A device according to claim 9, wherein at least one arrayed polynucleotide has a second polynucleotide hybridised thereto.
- 11 A device according to claim 9, wherein the arrayed polynucleotide is of known sequence.
- 12 A device according to claim 1, wherein the molecules are peptides or proteins.
- 13 A device comprising a high density array of relatively short molecules and relatively long polynucleotides immobilised on the surface of a solid support, wherein the polynucleotides are at a density that permits individual resolution of those parts thereof that extend beyond the relatively short molecules.

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- 14 A device according to claim 13, wherein the relatively short molecules are polynucleotides.
- 15 A device comprising an array of polynucleotide molecules immobilised on a solid surface, wherein each molecule comprises a polynucleotide duplex linked via a covalent bond to form a hairpin loop structure, one end of which comprises a target polynucleotide, and the array has a surface density which allows the target polynucleotides to be individually resolved.
- 16 A method for producing a device comprising a high density array of molecules capable of interrogation and immobilised on a solid planar surface, wherein the array allows the molecules to be individually resolved by optical microscopy, and wherein each molecule is immobilised by covalent bonding to the surface, other than at that part of each molecule that can be interrogated, the method comprising dispensing a solution comprising a mixture of molecules onto a solid surface under conditions that permit immobilisation and that minimise aggregation of the molecules in solution.
- 17 A method for producing a device comprising a high density array of polynucleotide molecules capable of interrogation and immobilised on a solid planar surface, wherein the array allows the polynucleotide molecules to be individually resolved by optical microscopy, and wherein each polynucleotide molecule is immobilised by covalent bonding to the surface, other than at that part of each polynucleotide molecule that can be interrogated, the method comprising
- (i) immobilising primer polynucleotides at discrete sites on the surface of a solid support, and
 - (ii) contacting the immobilised primers with target polynucleotides under hybridising conditions
- 18 A method for producing a device comprising a high density array of polynucleotide molecules capable of interrogation and immobilised on a solid planar surface, wherein the array allows the polynucleotide molecules to be individually resolved by optical microscopy, and wherein each polynucleotide molecule is immobilised by covalent bonding to the surface, other than at that part of each molecule that can be interrogated, the method comprising

- (i) immobilising first polynucleotides at discrete sites on the surface of a solid support, and hybridising thereto second polynucleotides which form single-stranded overhangs,
- (ii) contacting the product of step (i) with target polynucleotides under hybridising conditions;
- (iii) ligating the target polynucleotides to the first polynucleotides with a DNA ligase, and, optionally,
- (iv) removing the second polynucleotides

19 A method for the preparation of a device comprising an array of polynucleotide molecules immobilised on a solid surface, wherein each molecule comprises a polynucleotide duplex linked via a covalent bond to form a hairpin loop structure, one end of which comprises a target polynucleotide, and the array has a surface density which allows the target polynucleotides to be individually resolved, the method comprising ligating a target polynucleotide to the 5' end of a first molecule capable of forming said duplex, and immobilising the first molecule to the solid surface either before or after ligation.

20 A method according to claim 19, wherein immobilisation is after the ligation of the target polynucleotide.

21 A method according to claim 19, wherein the target polynucleotide is in the form of double-stranded DNA, ligation is between one strand of the DNA and the first molecule, and the other strand is removed after ligation

22 A method according to claim 21, wherein a further polynucleotide is hybridised to the first molecule with a one or more base gap between the further polynucleotide and the 3'-end of the first molecule, ligation is between the double-stranded DNA and the 5'-end of the first molecule and the further polynucleotide and hybridisation is subsequently disrupted to remove the further polynucleotide to form the target polynucleotide.

23 A method according to claim 19, wherein the 5'-end of the first molecule is phosphorylated and the target polynucleotide is dephosphorylated prior to ligation.

24 A method for forming a spatially addressable array, which comprises determining the sequences of a plurality of polynucleotide molecules immobilised on a device comprising a high density array of molecules capable of interrogation and immobilised on a solid planar surface, wherein the array allows the molecules to be individually

resolved by optical microscopy, and wherein each molecule is immobilised by covalent bonding to the surface, other than at that part of each molecule that can be interrogated

25 A method according to claim 24, further comprising the step of hybridising a polynucleotide molecule to its immobilised complement on the array.

5 26. A method according to claim 24, comprising the repeated steps of reacting the immobilised polynucleotide with a primer, a polymerase and the different nucleotide triphosphates under conditions sufficient for the polymerase reaction to proceed, wherein each nucleotide triphosphate is conjugated at its 3' position to a label capable of being characterised optically, determining which label (and thus which nucleotide) has
10 undergone the polymerisation reaction, and removing the label.

27 A method for characterising a plurality of first molecules, comprising contacting, under suitable conditions, a spatially addressed array of second molecules with the first molecules, and detecting a binding event, wherein the array comprises a high density array of molecules capable of interrogation and immobilised on a solid planar surface,
15 wherein the array allows the molecules to be individually resolved by optical microscopy, and wherein each molecule is immobilised by covalent bonding to the surface, other than at that part of each molecule that can be interrogated

28 A method according to claim 27, wherein the first molecules comprise a detectable tag

20 29. A method according to claim 28, wherein the tag is a fluorophore

30 A method according to claim 28, wherein the tag is a polynucleotide.

31 A method for characterising an organism, comprising the steps of contacting a defined array of polynucleotide molecules immobilised on a solid support with a plurality of fragments of the organism's genomic DNA, under hybridising conditions, and detecting
25 any hybridisation events, to obtain a distinct hybridisation pattern, wherein the array is comprising a high density array of molecules capable of interrogation and immobilised on a solid planar surface, wherein the array allows the molecules to be individually resolved by optical microscopy, and wherein each molecule is immobilised by covalent bonding to the surface, other than at that part of each molecule that can be interrogated

30 32. A method according to claim 31, wherein the organism is human

33. A method according to claim 31, wherein the organism is bacterial or viral

34. A method according to claim 31, wherein the fragments of genomic DNA are detectably-labelled.

35. A method for determining a single nucleotide polymorphism present in a genome, comprising

5 (i) immobilising fragments of said genome onto the surface of a solid support to form an array of polynucleotide molecules capable of interrogation, wherein the array allows the molecules to be individually resolved by optical microscopy, and wherein each molecule is immobilised by covalent bonding to the surface, other than at that part of each molecule that can be interrogated;

10 (ii) identifying nucleotides at selected positions in the genome, and
(iii) comparing the results of step (ii) with a known consensus sequence, and identifying any differences between the consensus sequence and said genome.

15 36. A method for determining a single nucleotide polymorphism present in a genome, comprising

(i) immobilising fragments of said genome onto the surface of a solid support to form an array of polynucleotide molecules capable of interrogation, wherein the array allows the molecules to be individually resolved by optical microscopy, and wherein each molecule is immobilised by covalent bonding to the surface, other than at that part of each molecule that can be interrogated;

20 (ii) contacting the array with each of the bases A, T, G and C, under conditions that permit the polymerase reaction to proceed and thereby form sequences complementary to those in the array,

25 (iii) determining the incorporation of a base at each of selected positions in the complementary sequences;

(iv) optionally repeating steps (ii) and (iii); and

30 (v) comparing the result of step (iii) with a known consensus sequence, and identifying any differences between the consensus sequence and said genome

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37 A method according to claim 36, wherein step(ii) is carried out by first contacting the array with three of the bases under conditions that permit the polymerase reaction to proceed, removing unreacted bases from the array and incorporating the remaining base, so that step (iii) proceeds only after incorporation of the remaining base

5 38 Use of a device comprising a high density array of polynucleotide molecules capable of interrogation and immobilised on a solid planar surface, wherein the array allows the molecules to be individually resolved by optical microscopy, and wherein each molecule is immobilised by covalent bonding to the surface via the 5' terminus, the 3' terminus, or via an internal nucleotide, for the capture of a second polynucleotide molecule capable of hybridising with the arrayed polynucleotide, comprising bringing into
10 contact with the device a sample containing or suspected of containing the second polynucleotide molecule, under hybridising conditions.

39 Use according to claim 38 wherein the sample is removed from contact with the device, thereby separating from the sample said second polynucleotide hybridised to an
15 arrayed polynucleotide

40 Use of a device comprising a high density array of molecules capable of interrogation and immobilised on a solid planar surface, wherein the array allows the molecules to be individually resolved by optical microscopy, and wherein each molecule is immobilised by covalent bonding to the surface, other than at that part of each
20 molecule that can be interrogated, for monitoring an interaction with a single molecule, comprising resolving an arrayed molecule with an imaging device.

41 Use according to claim 40, wherein the arrayed molecule undergoes repeated interactions with each interaction being monitored

42 Use of a device comprising an array of molecules immobilised on a solid surface,
25 wherein each molecule comprises a polynucleotide duplex linked via a covalent bond to form a hairpin loop structure, one end of which comprises a target polynucleotide, and the array has a surface density which allows the target polynucleotides to be individually resolved, in an analysis procedure to determine the sequence of the target polynucleotide.

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